

# Investigation on the Inheritance of Strain Specific Resistance to *Erwinia amylovora* in an Apple Rootstock Segregating Population

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## Abstract

*Erwinia amylovora* is the causative agent of apple rootstock blight. This disease is becoming more important as more susceptible and yet desirable scion cultivars are utilized in production using susceptible rootstocks. Utilization of disease resistant apple rootstocks increases the survivability of young trees infected by fire blight. Previous experiments in our breeding program showed that some apple rootstocks that derive their resistance from *Malus × robusta* cv. Robusta 5 show differential susceptibility to differentially virulent strains of *E. amylovora*. The goal of this experiment was to expand knowledge about the inheritance of the differential susceptibility in a mapping progeny of 170 individuals of the cross between apple rootstocks 'Ottawa 3' and 'Robusta 5'. Buds from each individual in the segregating population were grafted onto seedling rootstocks and trained to a single actively growing shoot. The shoots were inoculated at the same time with a differentially virulent strain of *E. amylovora*, E2002a and a virulent strain, Ea273. The same segregating population is being used to construct a genetic map with the intention of mapping any quantitative trait loci involved in the resistance. The Geneva rootstock breeding program has developed several new rootstocks that exhibit disease resistance to *E. amylovora* but a few of them have shown some susceptibility to differentially virulent strains. The results from this experiment will help us develop more durable resistance to rootstock fire blight.

## INTRODUCTION

The fire blight disease is caused by *Erwinia amylovora*, an anaerobic, gram-negative bacterium and its effects can be seen in blossoms, shoots and woody tissue of apple, pear and certain other rosaceous genera. This bacterial disease was widely spread throughout North America in the 1900s (Aldwinckle and Beer, 1979) and has also spread to most European apple growing countries and to other parts of the world (Jock et al., 2002). The rootstock phase of the disease can be very devastating as the bacteria that reach a susceptible rootstock have the ability to girdle and kill the whole tree. Unfortunately, as the disease spreads, its devastating effects are compounded by the widespread use of highly susceptible dwarfing rootstocks (Malling 9 and Malling 26) which are preferred over others because of their ability to increase productivity of high density orchards. The infection of a rootstock can occur in young shoots of rootstock suckers but more often from infection of scion blossoms followed by internal movement of the bacteria to the rootstock (Momol et al., 1998). Infection of the rootstock can also occur from wounds caused by insects (borers) and mechanical injury. Streptomycin

sprays can prevent some of the phases of the disease of the scion but they are not an effective control of rootstock blight (Norelli et al., 2003). Genetic resistance to *E. amylovora* has been observed in wild apple species and some cultivated varieties (Aldwinckle, 1974; Aldwinckle et al., 1974; Forsline et al., 2002; Gardner, 1977; Gardner et al., 1980). Gardner (1977) described the resistance derived from *Malus × robusta* cv. Robusta 5 as quantitative in nature but recent reports (Peil et al., 2007) indicate that a major factor (locus) is responsible for the resistance.

The purpose of this research was to learn about the inheritance of resistance described by Gardner (1977), utilizing two isolates of the causal bacteria *E. amylovora* (E2002a and Ea273) and to identify possible reasons for differential susceptibility to the two strains. The results obtained in this study will be used in combination with molecular genetic map to locate the factors segregating in this population and to identify DNA markers linked to the resistance genes to *E. amylovora*. This paper is a preliminary report of this research.

## MATERIALS AND METHODS

### Bacterial Isolates

Two strains of *E. amylovora*, E2002a and Ea273, were grown in Kado broth at 28°C. These cultures were then diluted with 0.05 M potassium phosphate, pH 6.5 to  $1 \times 10^6$  cfu/ml. These solutions were placed on ice and used for inoculation in the greenhouse within 2 hours of the dilution (Norelli et al., 2002).

### Greenhouse Inoculation

Rooted apple rootstock seedling liners were planted in bullet tubes in a greenhouse at the Geneva Experiment Station (NY). These seedling rootstocks received two buds from each of 192 different lines of a segregating population derived from a cross between Ottawa 3 and Robusta 5. When healed the buds were allowed to break by topping the seedling rootstock above the grafts and were trained to a single vigorously growing shoot. Six replications for each line/strain combinations (total of 2,304 plants) were randomized in six blocks (benches) and inoculated according to published protocols (Norelli et al., 2002). Briefly, rootstock inoculations were performed on specimens that had healthy, actively growing shoots. Scissors were dipped into the bacterial inoculum and used to cut the tips of soft young leaves close to the apical meristem, dipping the scissors into the bacterial culture between each cut. Percent lesion (ratio between the size of the necrotic lesion caused by the infection and the overall size of the primary shoot of the rootstock) was recorded 4–5 weeks after inoculation, when the lesions had ceased extending. The length of the lesion was observed by cutting the outer epidermal layer of the branch until a clear boundary between the healthy green tissue and brownish discolored infected tissue became visible. Data were collected on status of apical meristem (actively growing or not). Percent lesion data were analyzed using the PROC MIXED procedure of the SAS system software using a split plot design. Least square means were calculated for main effects (Line and Strain) and interactions (Line x Strain).

## RESULTS

Strain E2002a was more aggressive than strain Ea273. Even though there was a significant strong correlation (Pearson's 0.481  $P=0.001$ ) between the reaction of the two strains, there were some lines that showed more susceptibility to strain Ea273 (Fig. 3). This was also evident in the significant Strain x Line interaction in the Mixed Models analysis ( $P=0.0001$ ). The variable describing the status of the meristem (actively growing or not) had a significant effect in the model ( $P=0.0001$ ) but the total length of the shoot was not statistically significant. The distribution of the percent lesion means and variances of the lines for the whole population was different for the two strains (Fig. 1). With strain E2002a, 47 lines had no detectable lesions while with strain Ea273, 147 had none.



## DISCUSSION

We were led to this experiment because in earlier experiments we noticed that some elite rootstock breeding lines selected for fire blight resistance within our program were showing differential susceptibility to several isolates of *E. amylovora* (Fazio et al., 2006). Many of these breeding lines were progenies of 'Ottawa 3' × 'Robusta 5' crosses that had been selected with strain Ea273. Although Gardner (1977) described fire blight resistance derived from *Malus* × *robusta* cv. Robusta 5 as a quantitative character, there is mounting evidence (Peil et al., 2007) that a single major factor is segregating in progenies of this source. If we were to infer the inheritance of the 'Robusta 5' resistance strictly on the basis of the distribution of resistant individuals in the population we would arrive at two different conclusions based on the strain analyzed. It is possible that there is a major factor segregating, but there is strong evidence of interactions with multiple other factors that are segregating in this population. Further work is being performed using a quantitative model to elucidate the strain specific response, to characterize resistance to fire blight in other apple species and to discover new sources of resistance in exotic germplasm (Forsline et al., 2002).

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## Figures

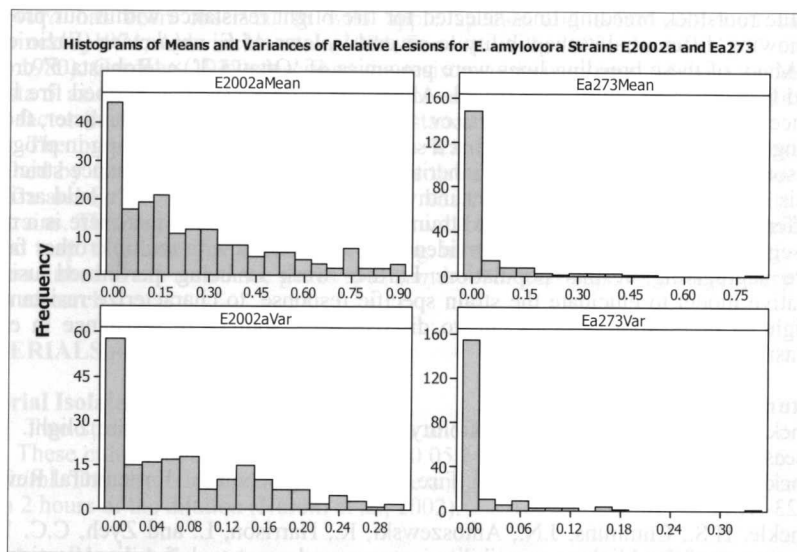


Fig. 1. Histogram of means and variances for the Percent Lesion variable (ratio between the size of the necrotic lesion caused by the infection and the overall size of the primary shoot of the rootstock) for lines in the 'Ottawa 3'  $\times$  'Robusta 5' segregating population.

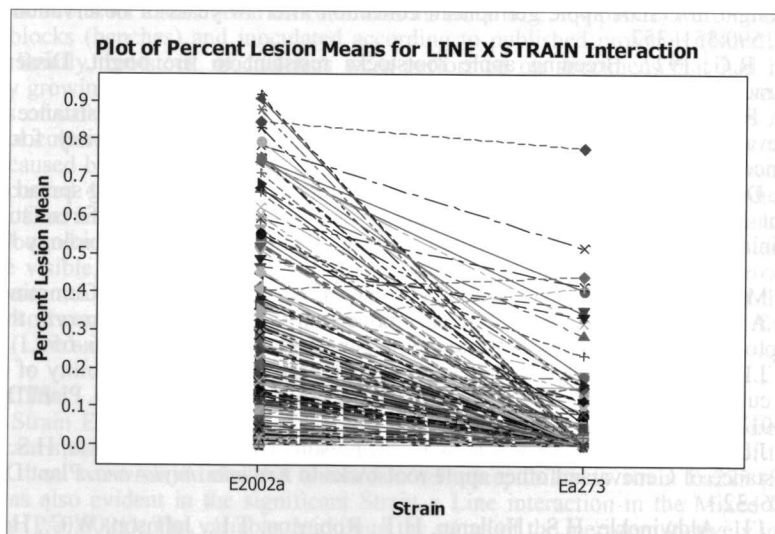


Fig. 2. Interactions plot of Percent Lesion means for the Line  $\times$  Strain Interaction. Strain Ea273 seems to be less virulent overall; however, it has the ability in some segregating O3  $\times$  R5 lines to elicit a more compatible response than the more virulent strain E2002a.

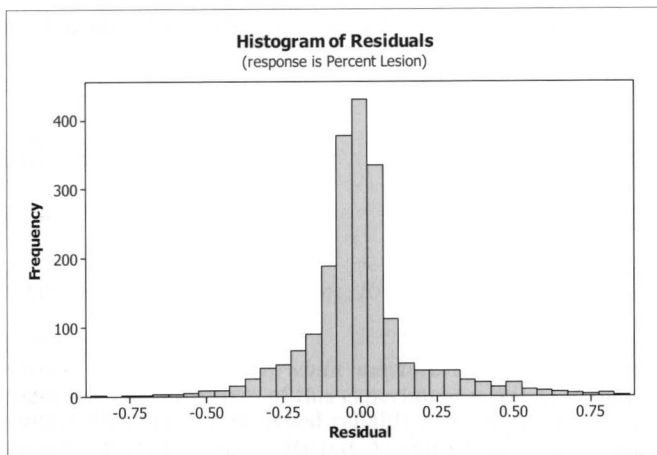


Fig. 3. Residuals for the analysis of the Percent Lesion variable are normally distributed narrowly around zero for this segregating population.

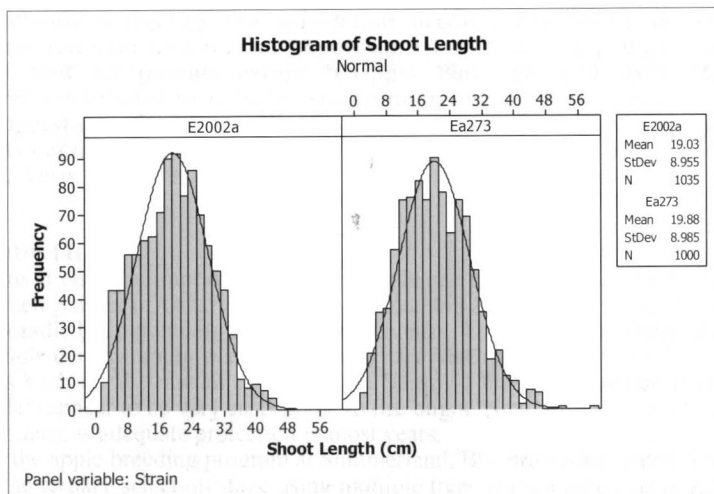


Fig. 4. The shoot length is an important variable to consider when making the measurement of Percent Lesion. The distributions of shoot length were normal and comparable for the two strains (Ea273, E2002a) giving confidence results of the comparison.